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Conditioned Fear Extinction and Generalization in Post-Traumatic Stress Disorder

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14. ABSTRACT

Post-traumatic stress disorder (PTSD) can affect an individual following exposure to a traumatic event. The exposure to trauma can evoke intense physical and emotional responses. Psychophysiological symptoms of PTSD can include an enhanced startle response; an effect that may result from an inability to inhibit fear. Conditioned fear can be measured using paradigms such as fear conditioning and fear extinction. Fear-potentiated startle is the process by which an individual's acoustic startle response is enhanced upon presentation of a conditioned stimulus (e.g., a colored shape) that was paired with an unpleasant unconditioned stimulus (e.g., an aversive airblast to the throat).

We have analyzed fear-processing in a population of PTSD patients from recent conflicts in the Middle East and healthy volunteers. One colored shape served as the reinforced conditioned stimulus (CS+, danger) and another colored shape served as the nonreinforced condition stimulus (CS-, safety). A 140 p.s.i airblast to the throat was used as the unconditioned stimulus. Subjects were fear-conditioned and, after a 10 minute interval, the subjects were trained to extinguish the fear. PTSD patients from the OIF theaters displayed greater fear-potentiated startle to the safety cue as well as delayed extinction of fear-potentiated startle in comparison to the healthy volunteers. In addition, combat veterans with PTSD report less discrimination between cues that are similar in nature to the danger cue.

15. SUBJECT TERMS
Posttraumatic stress disorder (PTSD); fear conditioning; extinction; startle response

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Section I: Introduction

This project represents an investigation of fear extinction and generalization in combat veterans returning from the theater of combat as part of the Global War on Terror (e.g., Operation Iraqi Freedom and Operation Enduring Freedom). We are studying fear processing in PTSD patients by examining (1) how well PTSD patients can *extinguish* learned fear and (2) the extent to which PTSD patients *generalize* their fear of specific trauma-related cues when exposed to similar cues. Reduced fear extinction and stimulus *over*-generalization may represent risk factors for PTSD and, as such, may still be present in PTSD patients and evident experimentally. A primary psychophysiological tool that we are using to assess fear extinction and stimulus generalization is *fear-potentiated startle* (FPS), or the relative increase in the amplitude of the acoustic startle response when a participant sees a signal that predicts the aversive stimulus used in this project. A third objective of this three-year study is to probe potential genetic biomarkers that govern one's resilience versus risk for developing PTSD following combat trauma. Using our established conditioned fear extinction paradigm (e.g., Norrholm et al., 2006; Norrholm et al., 2008; Norrholm et al., 2010a; Norrholm et al., 2010b), we are investigating potential candidate genetic polymorphisms underlying the vulnerability and symptomatology of PTSD. This translational study will focus on the contribution of genetic differences in PTSD patients and healthy control subjects to their individual ability to discriminate between danger and safety cues. In addition, this study will also examine the genetic contribution to the tendency for PTSD patients to over-generalize between danger cues and related stimuli (e.g., combat tones vs. environmental noises). In summary, an improved understanding of the genetic and epigenetic mechanisms that underlie the risk and symptoms associated with combat PTSD will enable clinicians to tailor treatment strategies according to the individual needs of each soldier returning from combat.

Section II: Body

Several major tasks at outlined in the approved Statement of Work have been accomplished in this period. The major milestone of data collection for the fear acquisition and extinction task (SOW Task 2) has been completed. Data collection for stimulus generalization (SOW Task 3) was ongoing throughout the previous year and is in its final stages. Genetic assays (SOW Task 4) are ongoing at Emory University. This CDMRP/DoD-funded project was recently awarded an additional year of funding to further examine the utility of a recently developed retrieval + extinction paradigm aimed at facilitating conditioned fear extinction in rodent and human models (SOW Task 5). This novel paradigm represents a novel, non-pharmacological avenue to explore for extinction-based therapies for PTSD and related anxiety disorders. The major task of statistical analysis, data interpretation, and manuscript preparation (SOW Task 6) is ongoing. An Extension Without Funds (EWOF) is pending to complete SOW Tasks 5 and 6.

Data Collection: Fear Acquisition and Fear Extinction Paradigm (Task 2)

This task involved recruiting, screening, clinically assessing, and testing OIF/OEF veterans. The clinical assessments included diagnostic interviews in order to divide the veterans into groups according to their status: No PTSD diagnosis, PTSD diagnosis, or

Depression diagnosis. The subjects were then tested using the psychophysiological methods described in the original funding proposal. Briefly, fear-potentiated startle responses with electromyographic recordings of the eyeblink muscle was assessed using a colored shape as the reinforced conditioned stimulus (CS+, danger) and another colored shape as the nonreinforced condition stimulus (CS-, safety). A 140 p.s.i airblast to the throat served as the unconditioned stimulus. Subjects were fear-conditioned and, after a 10 minute interval, the subjects were trained to extinguish the fear. A trial-by-trial measure of awareness of reinforcement contingencies was collected via a response pad.

Forty-three subjects participated after signing an informed consent form approved by the Emory University Institutional Review Board and the Atlanta VAMC Research and Development Committee. OIF veterans were referred to the study from the Trauma Recovery Program and related medical clinics at the Atlanta VAMC and healthy volunteers were recruited from the Emory University community. Using strict criteria for matching test groups on factors such as mental health status and trauma history, 43 subjects were included in fear extinction analyses: PTSD (n=25) and No PTSD (n=18).

As shown in Figure 1, robust fear-potentiated startle to the danger cue (cue A) was displayed in both participants with and without PTSD (Repeated Measures ANOVA, $F(1,35) = 12.06$, $p = 0.001$. There was no significant difference between the no PTSD and PTSD groups.

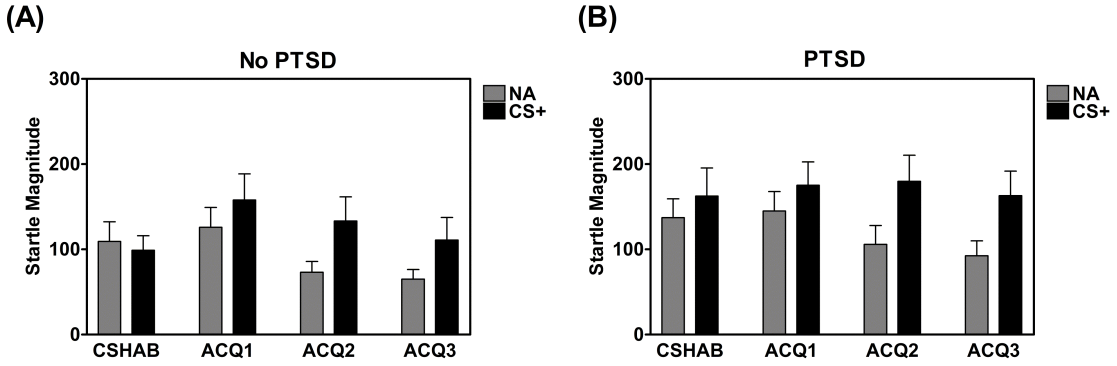

Figure 1. Both the (A) No PTSD and (B) PTSD groups displayed robust fear-potentiated startle to the reinforced conditioned stimulus (CS+) as compared to startle responses to the startle probe alone (NA). Repeated Measures ANOVA, main effect of Trial Type, $F(1,35) = 12.56$, $p = 0.001$, no significant between-group difference.

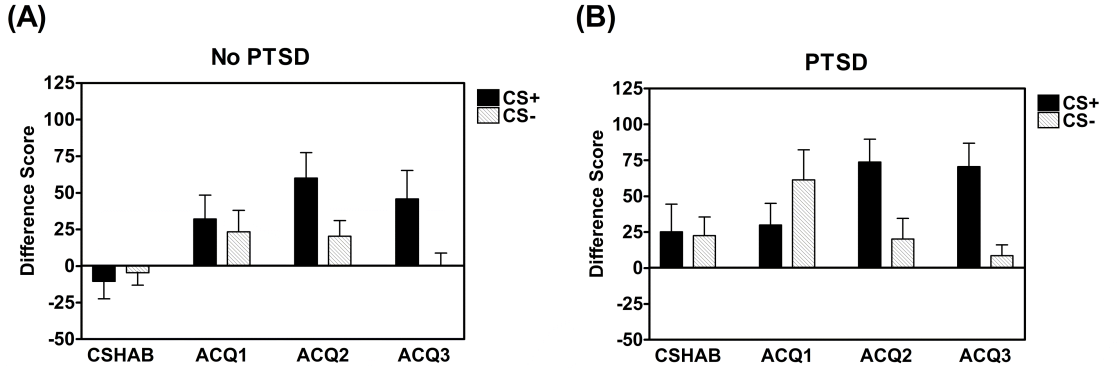

Figure 2. Both the (A) No PTSD and (B) PTSD groups displayed significant discrimination between the reinforced conditioned stimulus (CS+) and the nonreinforced CS- during the Fear Acquisition session. Repeated Measures ANOVA, significant Trial x Trial Type interaction, $F(1,41) = 89.51$, $p < 0.001$, no Group x Trial x Trial Type interaction and no significant between-group difference.

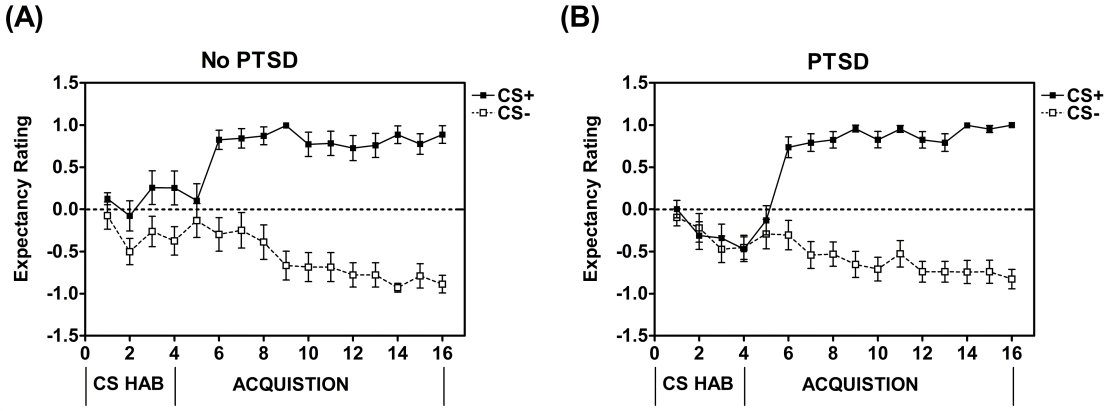

Figure 3. Both the (A) No PTSD and (B) PTSD groups showed significant discrimination between the reinforced CS+ and the nonreinforced CS- during Fear Acquisition based on trial-by-trial US expectancy responses.

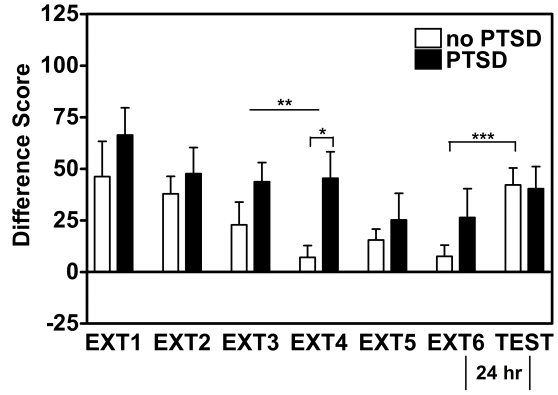

Figure 4. Controls (no PTSD) showed significant within-session extinction from Blocks 1 to 4 (Repeated Measures ANOVA, significant effect of BLOCK, $F(1,18) = 5.54$, $p = 0.03$) whereas combat PTSD patients did not show within-session extinction during these early Blocks of extinction (Repeated Measures ANOVA, no significant effect of BLOCK, $F(1,24) = 2.06$, $p = 0.16$). Combat veterans with PTSD showed elevated levels of fear-potentiated startle (i.e., significantly less extinction of fear) during the middle stages of the extinction session (Blocks 3 and 4) as compared to psychiatrically healthy controls without PTSD (Repeated Measures ANOVA, Between-subjects effect, $F(1,42) = 5.72$, $p = 0.02$). The divergent levels of fear extinction observed in the controls versus PTSD patients were most evident in Block 4 of extinction; the controls were at a terminal extinction level at this point whereas the degree of fear extinction in the PTSD patients remained elevated (One-way ANOVA, $F(1,42) = 5.77$, $p = 0.02$)

We have previously shown that traumatized civilians with PTSD exhibit extinction deficits in the early to middle stages of the extinction training session (see Norrholm et al., 2010a). As such, we hypothesized that we would observe similar effects in the current combat PTSD population. Both the psychiatrically healthy control group and OIF/OEF veterans with PTSD displayed significant within-session extinction across all six blocks of the extinction session. The control group (no PTSD) showed significant within-session extinction from Blocks 1 to 4, whereas the PTSD group did not show within-session extinction during these early Blocks of extinction. The PTSD group

showed less extinction of fear during the middle stages of the extinction session (Blocks 3 and 4) compared to the no PTSD group. The difference between controls and PTSD patients was most evident in Block 4 of extinction. The no PTSD group showed robust spontaneous recovery whereas the PTSD group did not show significant spontaneous recovery (Extinction Block 6 versus Test).

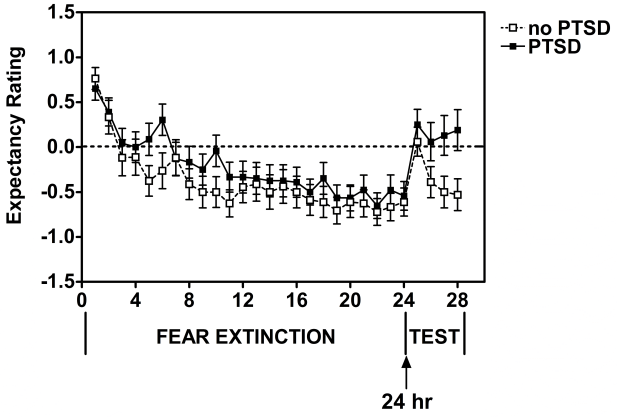

Figure 5. Participants with and without PTSD displayed a significant reduction in ratings of DANGER to the previously reinforced CS+ during the Fear Extinction phase (Repeated Measures ANOVA, $F(1,41) = 70.31$, $P < 0.001$). There was no group difference between individuals with and without PTSD when comparing within-session extinction of DANGER ratings. Both the control and PTSD groups showed a significant increase in US-expectancy ratings upon presentation of the previously reinforced CS+ during the Extinction Recall (Test) session (Repeated Measures ANOVA, $F(1,33) = 13.48$, $p = 0.001$). However, there was a significant difference between the no PTSD and PTSD groups with respect to their responses to the previously reinforced/extinguished CS+ during the Extinction Recall (Test) session (Repeated Measures ANOVA, $F(1,33) = 5.32$, $p = 0.03$). Controls showed a significant decrease in their US expectancy ratings during the four trials of the Test session whereas PTSD patients did not.

Both the psychiatrically healthy (no PTSD) and PTSD groups showed a significant decrease in ratings of danger upon presentation of the previously reinforced CS+ during the Fear Extinction session. Both the control and PTSD groups showed a significant increase in US-expectancy ratings upon presentation of the previously reinforced CS+ during the Extinction Recall (Test) session. However, there was a significant difference between the no PTSD and PTSD groups with respect to their responses to the previously reinforced/extinguished CS+ during the Extinction Recall (Test) session. Controls showed a significant decrease in their US expectancy ratings during the four trials of the Test session whereas PTSD patients did not.

An inability to inhibit learned fear under conditions of safety can underlie several PTSD symptoms, most notably re-experiencing (Friedman 2006; Norrholm & Jovanovic 2010). Our group has previously shown fear inhibition deficits in response to safety cues in both civilian (Jovanovic et al. 2010a) and combat PTSD patients (Jovanovic et al. 2009). In addition, we recently reported fear extinction deficits in traumatized civilians with PTSD (Norrholm et al. 2010b). In the present study, we expanded our prior investigations of fear extinction in PTSD using a fear-potentiated startle paradigm previously characterized and replicated (Norrholm et al. 2006; Norrholm et al. 2010a; Norrholm et al. 2008). To summarize, the primary findings of our Fear Extinction study are: (a) both combat veterans with PTSD and psychiatrically healthy controls displayed robust fear-potentiated startle to the CS+ and significant discrimination between the CS+ and CS- during Acquisition, (b) both combat veterans with PTSD and controls correctly

identified the CS+ and CS- as DANGER and SAFETY cues, respectively, during Fear Acquisition, (c) PTSD patients displayed impaired within-session extinction of fear-potentiated startle during the early phases of the Fear Extinction session, (d) both PTSD patients and controls showed spontaneous recovery to the previously reinforced and subsequently extinguished CS+ during the Extinction Recall session with the Control group showing more robust recovery, and (e) PTSD patients remained uncertain about the safety of the CS+ during the Extinction Recall phase as compared to healthy controls.

Data Collection: Stimulus Generalization Paradigm (SOW Task 3)

This task involved developing a new fear-potentiated startle paradigm in which the conditioned stimuli were auditory rather than visual cues. The psychophysiological data recording methods were the same as described above, using the EMG of the startle response. The auditory cues were selected from a range of pure tones (250, 500, 1000, 2000, 4000, 6000, 8000 Hz). Subjects were differentially conditioned to the highest and lowest tones such that trials containing the 8000 Hz tone (CS+) were reinforced with an airblast while tones at 250 Hz were not reinforced (CS-). This allows us to examine the ability of PTSD patients to inhibit fear to the nonreinforced cue (CS-) as well as the extent to which their fear is generalized to tones that are similar in frequency to the reinforced cue (8000 Hz, CS+). The reinforced (CS+) and nonreinforced tones (CS-) were counterbalanced across participants.

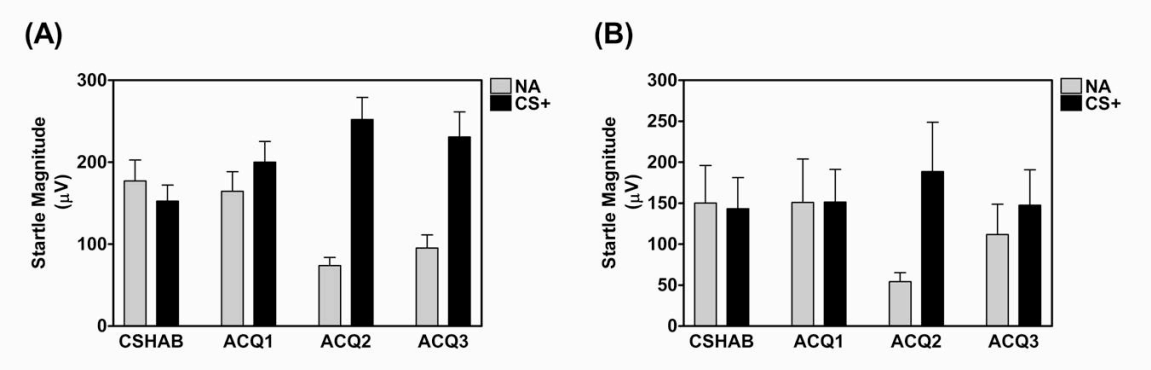

Figure 6. During Fear Acquisition, psychiatrically healthy controls (**panel A**) and OIF/OEF veterans with PTSD (**panel B**) showed robust fear-potentiated startle to the CS+ (Repeated Measures ANOVA, Significant Block x Trial Type interaction, $F(1,82) = 12.5$, $p = 0.001$, no Group x Block interaction or between-group effect).

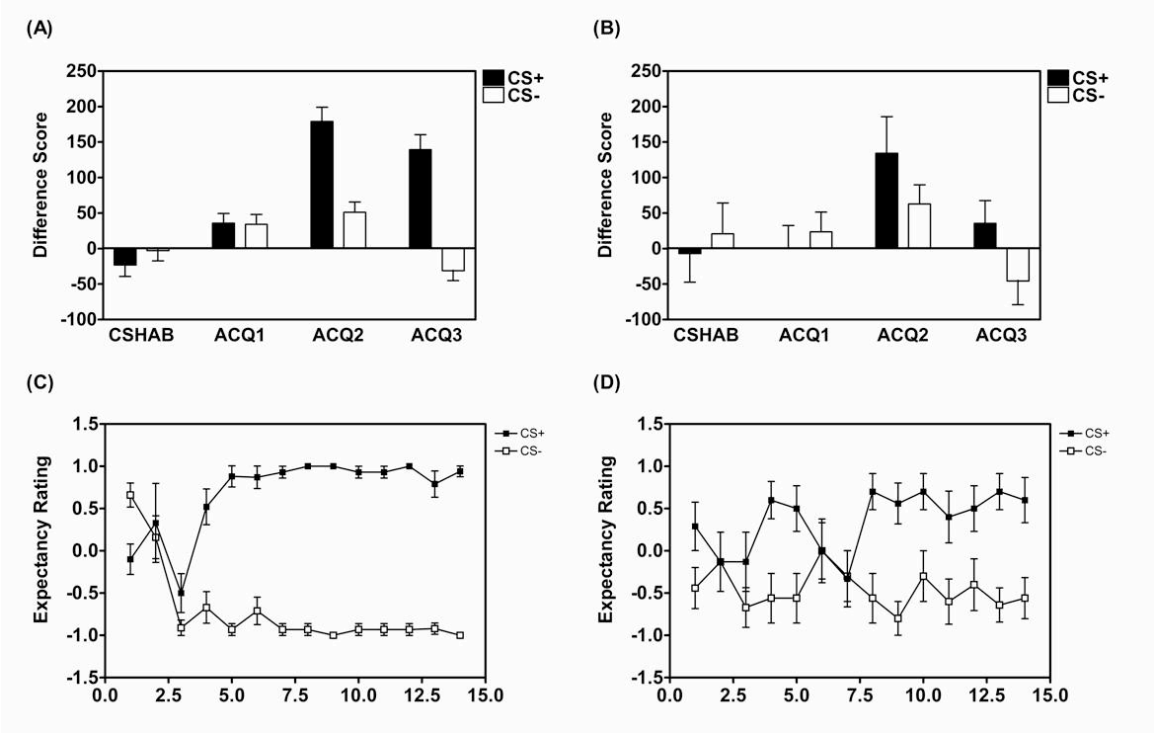

Figure 7. Psychiatrically healthy controls (**panel A**) and combat veterans with PTSD (**panel B**) showed significant discrimination between the CS+ and CS- both in fear-potentiated startle (Repeated Measures ANOVA, Significant Block x Trial Type interaction, $F(1,82) = 20.7$, $p < 0.001$, no Group x Block interaction or between-group effect) and US expectancy ratings (Controls: **panel C**, PTSD patients: **panel D**; Repeated Measures ANOVA, Significant Trial x Trial Type interaction $F(1,21) = 57.5$, $p < 0.001$, Group x Trial x Trial Type interaction, $F(1,21) = 15.7$, $p = 0.001$). Difference score = Startle magnitude in response to the CS – Startle Magnitude to the noise probe alone (baseline).

As shown in Figure 6, psychiatrically healthy controls and combat veterans with PTSD showed robust fear-potentiated startle to the CS+ during the Acquisition phase of the Stimulus Generalization paradigm. Both groups were also able to discriminate between the reinforced CS+ and non-reinforced CS- during Acquisition. However, there was a significant difference between the groups in terms of cognitive awareness of the CS-US association. The PTSD group (Figure 7, panel D), as compared to the control group (Figure 7, panel C) shows much less certainty of DANGER (coded as +1) and SAFETY (coded as -1) on CS+ and CS- trials, respectively. This is a surprising finding given that our previous data (see Figure 3 above) show that PTSD patients can accurately identify visual conditioned stimuli as dangerous or safe. We will further analyze this discrepancy between PTSD cohorts to determine if these observed differences are due to factors such as trauma type, symptom severity, or co-morbidity with major depression.

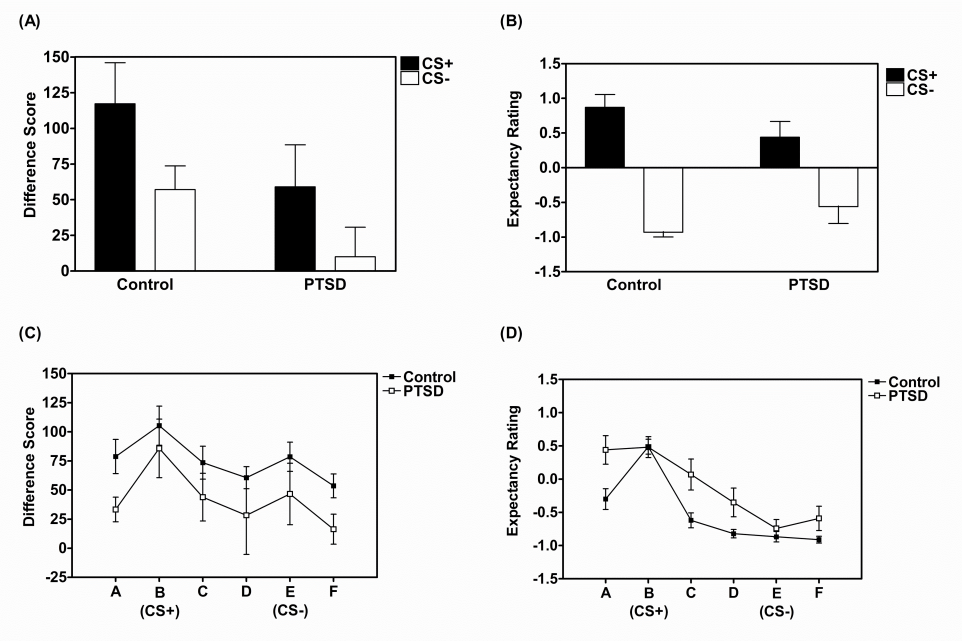

Figure 8. The Generalization test was preceded by a brief re-training phase consisting of 4 presentations each of the CS+, CS-, and noise probe alone. As shown in **panels A and B**, both groups continued to discriminate between the CS+ and CS- based on fear-potentiated startle (Repeated Measures ANOVA, Main Effect of Trial Type, $F(1,80) = 4.21$, $p < 0.05$, no significant Group x Trial Type interaction or between-group effect) and US-expectancy measures (Repeated Measures ANOVA, Main Effect of Trial Type, $F(1,40) = 96$, $p < 0.001$, no significant Group x Trial Type interaction or between-group effect**).** During the Generalization test, psychiatrically healthy participants and veterans with PTSD showed heightened startle responses to the tones that were closest in frequency to the CS+ (tone B) and less responding to the tones that were closest in frequency to the CS- (tone E). Based on fear-potentiated startle measures, there were no group differences between the control participants and PTSD patients with regard to their generalization gradients during the Generalization Test (**panels C and D**, Repeated Measures ANOVA, Main Effect of Block, $F(1,80) = 4.67$, $p = 0.03$, no Group x Block interaction or between-group effect). However, based on US-expectancy measures, combat veterans with PTSD show a more shallow generalization gradient as compared to healthy controls (**panel D**, Repeated Measures ANOVA, Main Effect of Block, $F(1,39) = 44.9$, $p < 0.001$, significant Group x Block interaction, $F(1,39) = 7.72$, $p = 0.008$, and Between-group effect, $F(1,39) = 14.4$, $p < 0.001$).

These data demonstrate a significant difference between OIF/OEF veterans with PTSD and healthy controls with regard to their cognitive reports of safety upon presentation of cues similar in nature to a previously reinforced (ie., dangerous) cue. The use of this type of generalization test has the potential to contribute to our understanding on PTSD symptoms with regard to generalization of fear responses. For example, a common clinical observation is a heightened fear response to environmental sounds (e.g., fireworks, doors slamming) that are similar to trauma-related cues (e.g., explosions, gunfire).

Data Collection: DNA Samples (SOW Task 4)

To date, we have analyzed 227 saliva samples for genotypic data. The genetic analyses have included several single nucleotide polymorphisms (SNPs) that have recently been shown to influence the expression and severity of anxiety disorders such as

PTSD. For example, a non-synonymous, SNP in the gene coding for steroid 5-α-reductase type 2 (*SRD5a2*) has been associated with reduced capacity for the enzymatic conversion of testosterone to dihydrotestosterone. Our group has shown that a functional variation within the androgen converting enzyme, *SRD5a2,* influences the severity of post-traumatic stress symptoms and risk for diagnosis of PTSD in civilians recruited from an urban, inner-city population. As part of the current CDMRP-funded study, we will assess the contribution of this SNP to combat-related PTSD.

In addition, our group has examined SNPs spanning the genes for pituitary adenylate cyclase activating polypeptide (PACAP; encoded by ADCYAP1) and one of its receptors (PAC1; encoded by ADCYAP1R1) in traumatized civilians (Ressler et al., 2011). We have shown that alterations in the PACAP-PAC1 pathway may be involved in abnormal stress responses underlying PTSD. As part of this CDMRP-funded study, we will assess the contribution of this SNP to the expression and severity of combat-related PTSD.

SNP	GENE	Frequency					
RS10491389	*EFNA5*	CT	7	TT	219		
RS110402	*CRHR1*	CC	74	CT	100	TT	52
RS1147198	*NTRK2*	AA	115	CA	89	CC	23
RS11982285	*ADCYAP1R1*	CC	211	TT	8		
RS1269637	*NRCAM*	AA	6	GA	50	GG	170
RS1360780	*FKBP5*	CC	102	TC	98	TT	24
RS1611115	*DBH*	CC	144	TC	68	TT	14
RS16147	*NPY*	AA	59	AG	117	GG	48
RS16855131	*DISC1*	CC	1	CT	11	TT	213
RS17733405	*PTPN14*	CT	24	TT	204		
RS17795692	*PDE4d*	AA	197	AG	24	GG	6
RS1799978	*DRD2*	AA	193	GA	31	GG	2
RS1867283	*NTRK2*	AA	27	AG	100	GG	100
RS1912674	*PDE10*	CC	18	CT	68	TT	130
RS2267735	*ADCYAP1R1*	CC	70	GC	108	GG	48
RS2268495	*OXTR*	AA	9	AG	93	GG	125
RS2301261	*OXTR*	AA	7	AG	61	GG	159
RS2522833	*PCLO*	AA	101	CA	92	CC	32
RS2717162	*GALR1*	CC	20	CT	104	TT	102
RS324420	*FAAH*	AA	14	CA	71	CC	142
RS3800373	*FKBP5*	GG	25	GT	100	TT	100
RS4606	*RGS2*	CC	109	CG	88	GG	28
RS4680	*COMT*	AA	44	GA	99	GG	83
RS4875113	*CSMD1*	AA	2	CA	20	CC	205
RS497	*NTS*	CC	113	CT	2		
RS523349	*SRD5A2*	CC	100	CG	102	GG	22
RS6265	*BDNF*	AA	5	GA	47	GG	174
RS6277	*DRD2*	CC	92	TC	92	TT	39

RS6675281	*DISC1*	CC	176	TC	47	TT	3
RS7195140	*GRIN2A*	CC	213	TC	14		
RS7209436	*CRHR1*	CC	77	CT	97	TT	52
RS9296158	*FKBP5*	AA	29	AG	101	GG	96
RS9470080	*FKBP5*	CC	88	CT	109	TT	28

Single nucleotide polymorphisms (SNPs), their associated genes, and the frequencies observed in the study population. These genes are of greatest interest with regard to the etiology of anxiety and stress disorders and will be examined as they relate to fear learning during our ongoing analyses of data. Genetic data have been analyzed for 227 consenting participants.

Data Collection: Administer the Retrieval + Extinction Paradigm (SOW Task 5)

This CDMRP/DoD-funded project was recently awarded an additional year of funding to further examine the utility of a recently developed reactivation paradigm aimed at facilitating conditioned fear extinction in rodent and human models. This novel paradigm represents a novel, non-pharmacological avenue to explore for extinction-based therapies for PTSD and related anxiety disorders. In the last year, we have implemented and optimized this paradigm in psychiatrically healthy volunteers. As a necessary first step, we examined the use of a reactivation paradigm in which participants are: (1) fear conditioned to 2 distinct conditioned stimuli (termed CSs; e.g., colored geometric shapes), (2) reminded of this conditioning to one of the CSs, (3) administered a fear extinction training session, and then (4) presented with a test to determine if conditioned fear has returned through spontaneous recovery or remains at an extinguished level. The following results have been found to date:

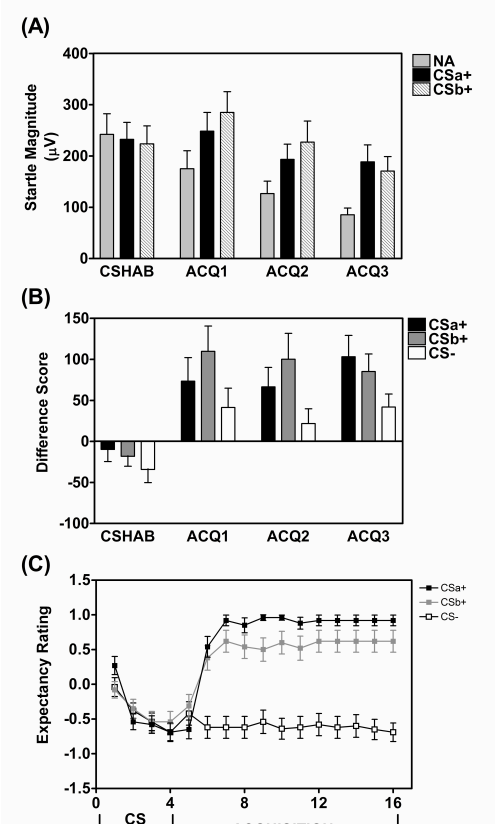

Figure 9. Psychiatrically healthy volunteers display Fear Acquisition to two distinct CSs as based on fear-potentiated startle measures (**panel A**; Repeated Measures ANOVA, Significant Block x Trial Type Interaction, $F(1,23) = 8.52$, $p = 0.008$). Psychiatrically healthy volunteers display Discrimination between two CS+'s and a CS- as based on fear-potentiated startle (**panel B**; CSa+ vs. CS-, Repeated Measures ANOVA, Main Effect of Trial Type, $F(1,23) = 19.04$, $p < 0.001$; CSb+ vs. CS-, Repeated Measures ANOVA, Main Effect of Trial Type, $F(1,23) = 18.81$, $p < 0.001$) and US-expectancy ratings (**panel C**; CSa+ vs. CS-, Repeated Measures ANOVA, Main Trial x Trial Type, $F(1,23) = 57.12$, $p < 0.001$. CSb+ vs. CS-, Repeated Measures ANOVA, Main Trial x Trial Type, $F(1,21) = 8.96$, $p = 0.007$).

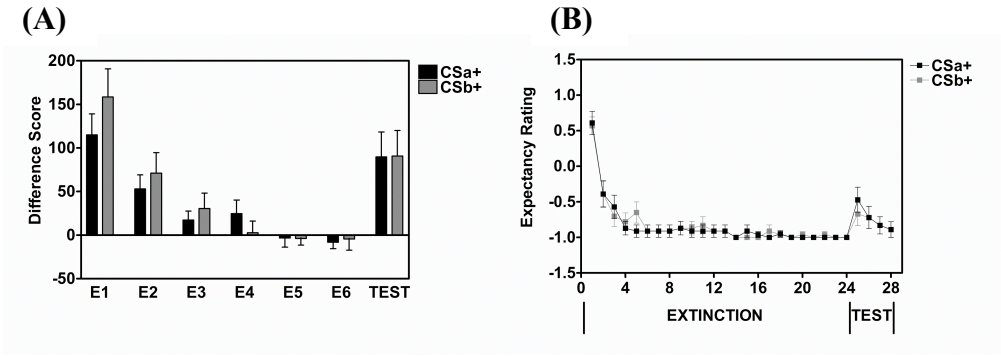

Figure 10. Psychiatrically healthy controls showed significant extinction of fear-potentiated startle (**panel A**; Repeated Measures ANOVA, Main Effect of Block, $F(1,20) = 21.20$, $p < 0.001$) and US-expectancy (**panel B**; Repeated Measures ANOVA, Main Effect of Trial, $F(1,21) = 67.76$, $p < 0.001$) responses to two distinct CSs during the Extinction Training phase. In addition, healthy controls showed spontaneous recovery of fear responses during the Extinction Test conducted 24 hours after Extinction Training (*Startle*: Repeated Measures ANOVA, Main Effect of Block, $F(1,14) = 11.5$, $p = 0.004$; *US-expectancy*: Repeated Measures ANOVA, Main Effect of Trial, $F(1,16) = 9.66$, $p = 0.007$).

Analyses and Evaluation (SOW Task 6)

A database containing the self-report, demographic, and psychophysiological data was created and statistical analyses have been an ongoing task of this research study. As listed below, seven manuscripts based on this work have been published to date. While we have done data analysis and interpretation as the tasks were being completed, analyses and publication of data will continue as part of a pending EWOF.

Section III: Key Research Accomplishments

The following research accomplishments are reported at this time: (1) the fear extinction protocol employed in this study is highly sensitive for the development of fear learning (via fear-potentiated startle measures) in both psychiatrically healthy individuals and combat veterans with PTSD, (2) this protocol also allows for the detection of danger and safety learning in the aforementioned populations, (3) the employment of this protocol has allowed us to demonstrate significant deficits in fear extinction in PTSD patients; an effect that is believed to underlie the fear-related PTSD symptom clusters of hyperarousal and re-experiencing, (4) the inclusion of cognitive awareness measures has illustrated that PTSD patients display a significant degree of uncertainty about the safety of fear-related cues even in the *absence* of aversive consequences, (5) the stimulus generalization paradigm employed in this study has detected a cognitive difference between psychiatrically healthy controls and OIF/OEF veterans with PTSD in the manner in which they perceive danger and safety when presented with cues of similar nature to a previously noxious cue, and (6) we have established a paradigm with three distinct conditioned stimuli (2 reinforced CS+'s and a non-reinforced CS-) that will allow us to employ a retrieval + extinction paradigm as a potential non-pharmacological

methodology for enhancing conditioned fear extinction.

Thus, the results of this study strongly suggest that the fear extinction and generalization protocols employed have high clinical relevance and potential as: (1) objective measures of fear excitation, inhibition, and generalization in recently returning combat veterans at the outset of treatment, (2) objective measures of "fear load" over the course of PTSD treatment, and (3) platforms for pre-clinical assessments of promising facilitators of extinction learning.

Section IV: Reportable Outcomes

Manuscripts

Jovanovic, T., Jambrošić Sakoman, A., Kozarić-Kovačić, D., Meštrović, A.H., Duncan, E.J., Davis, M., & **Norrholm, S.D.** (2012). Acute stress disorder vs. chronic posttraumatic stress disorder: inhibition of fear as a function of time since trauma. *Depression and Anxiety*.

Norrholm, S.D., Anderson, K.M., Olin, I.W., Jovanovic, T., Kwon, C., Warren, V.T., McCarthy, A.J., Bosshardt, L., Sabree, J., Duncan, E.J., Rothbaum, B.O., & Bradley, B. (2011). Versatility of fear-potentiated startle paradigms for assessing human conditioned fear extinction and return of fear. *Frontiers in Behavioral Neuroscience*.

Norrholm, S.D., & Jovanovic, T. (2011). Translational fear inhibition models as indices of trauma-related psychopathology. *Current Psychiatry Reviews*, 7(3): 194-204.

Jovanovic T., & **Norrholm, S.D.** (2011). Neural mechanisms of impaired fear inhibition in posttraumatic stress disorder. *Frontiers in Behavioral Neuroscience*, 5,44.

Norrholm, S.D., Olin, I.W., Jovanovic, T., McCarthy, A.J., Duncan, E.J., Skelton, K., & Bradley, B. (2010a). Conditioned fear extinction is impaired in combat veterans from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). *Proceedings of the 27th Army Science Conference*.

Norrholm, S.D. & Jovanovic, T. (2010). Tailoring therapeutic strategies for treating posttraumatic stress disorder symptom clusters. *Neuropsychiatric Disease and Treatment*, 2010(6), 517-532.

Norrholm, S.D., Jovanovic, T., Olin, I.W., Sands, L.A., Karapanou, I., Bradley, B., & Ressler, K.J. (2010b). Fear extinction in traumatized civilians with posttraumatic stress disorder: relation to symptom severity. *Biological Psychiatry,* 69(6):556-63.

Abstracts

Norrholm, S.D., Jovanovic, T., Anderson, K.M., Kwon, K., McCarthy, A.J., Ressler, K.J., & Bradley, B. (2012). Combat veterans from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) display impaired fear extinction and an over-generalization of fear responses. 28th Annual Meeting of the International Society for Traumatic Stress Studies, Los Angeles, CA.

Norrholm, S.D. (2011). Conditioned fear extinction and stimulus generalization as psychophysiological tools for assessing combat PTSD symptomatology. Symposium: Translational Methods in Anxiety Disorders Research: From Animals to Humans and Back Again. 31st Annual Meeting of the Anxiety Disorders Association of America, New Orleans, LA.

Norrholm, S.D., Olin, I.W., Jovanovic, T., McCarthy, A.J., Duncan, E.J., Skelton, K., & Bradley, B. (2010). Conditioned fear extinction is impaired in combat veterans from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). 27th Annual Army Science Conference, Orlando, FL.

Jovanovic, T., **Norrholm, S.D.**, Davis, M., Bradley, B., & Ressler, K. (2010). Fear acquisition and inhibition in posttraumatic stress disorder. Annual Meeting of the Pavlovian Society, Baltimore, MD.

Olin, I.O., Jovanovic, T., Skelton, K., Bradley, B., Duncan, E., & Ressler, K., & **Norrholm, S.D.** (2010). Conditioned fear extinction in combat and civilian traumatized populations with PTSD. Anxiety Disorders Association of America, 30th Annual Conference, Baltimore, MD.

Norrholm, S.D., Jovanovic, T., Skelton, K., Bradley, B., Duncan, E., & Ressler, K. (2009). Conditioned fear extinction in combat and civilian traumatized populations with PTSD. 48th Annual Meeting of the American College of Neuropsychopharmacology, Hollywood, FL.

Norrholm, S.D., Olin, I., Jones, M., Leimbach, L., Daugherty, M., Crowe, C., Skelton, K., Jovanovic, T., Ressler, K., Bradley, B., & Duncan, E. (2009). Conditioned Fear Acquisition, Discrimination, and Extinction in Combat Veterans from Operation Iraqi Freedom with Post-traumatic Stress Disorder. 3rd Annual Military Health Research Forum, Kansas City, MO.

Jones, M., Jovanovic, T., Olin, I., Daugherty, M., Skelton, K., Duncan, E., Bradley, B., & **Norrholm, S.D.** (2009). Conditioned fear acquisition, discrimination, and extinction in combat veterans from Operation Iraqi Freedom (OIF) with posttraumatic stress disorder (PTSD). Center for Behavioral Neuroscience (CBN) Summer Research Symposium, Atlanta, GA.

Leimbach, L.B., Daugherty, M.D., Russ, E., Crowe, C., Skelton, K., Jovanovic, T., Ressler, K., Duncan, E, Bradley, B., & **Norrholm, S.D.** (2009). Conditioned fear acquisition, discrimination, and extinction in combat veterans from Operation Iraqi Freedom (OIF) with posttraumatic stress disorder (PTSD). Georgia/South Carolina Neuroscience Consortium Annual Meeting, Athens, GA.

Daugherty, M.D., Leimbach, L.B., Russ, E., Crowe, C., Skelton, K., **Norrholm, S.D.** & Bradley, B. (2009). OIF/OEF veterans with post-traumatic stress disorder: A first look. Georgia/South Carolina Neuroscience Consortium Annual Meeting, Athens, GA.

Presentations

Jovanovic, T. & **Norrholm, S.D.** (2012). Impaired safety signal processing as a brain-based intermediate phenotype. Society for Psychophysiological Research, 52nd Annual Meeting, New Orleans, LA.

Invited Talk: **Norrholm, S.D.** (2012). Overcoming your fear load: translational keys to understanding posttraumatic stress disorder (PTSD) symptoms. PTSD Clinical Team, Ralph H. Johnson VA Medical Center/Medical University of South Carolina, Charleston, S.C.

Invited Talk: **Norrholm, S.D.** (2012). Conditioned fear extinction and fear inhibition as psychophysiological indices of trauma-related psychopathology. Family Violence and Sexual Assault Seminar, Department of Psychology, Northern Illinois University, DeKalb, IL.

Invited Talk: **Norrholm, S.D.**, & Jovanovic, T. (2012). A Mini-Symposium: "Insights on Trauma from Fear Learning in Humans." Department of Psychiatry and Behavioral Sciences, Stanford University, Palo Alto, CA.

Norrholm, S.D. (2011). Conditioned fear extinction and stimulus generalization as psychophysiological tools for assessing combat PTSD symptomatology. Symposium: Translational Methods in Anxiety Disorders Research: From Animals to Humans and Back Again. 31st Annual Meeting of the Anxiety Disorders Association of America, New Orleans, LA.

Norrholm, S.D., Olin, I.W., Jovanovic, T., McCarthy, A.J., Duncan, E.J., Skelton, K., & Bradley, B. (2010). Conditioned fear extinction is impaired in combat veterans from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). 27th Annual Army Science Conference, Orlando, FL.

Norrholm, S.D. (Fall 2010). Department of Defense, Congressionally Directed Research Programs (DoD CDMRP) Research Highlight Video.

Norrholm, S.D., Jovanovic, T., Leimbach, L., Bradley, B., & Duncan, E. (November 2009). Fear Extinction in Veterans From Operation Iraqi Freedom (OIF) with

Posttraumatic Stress Disorder. 25th Annual Meeting of the International Society for Traumatic Stress Studies, Atlanta, GA.

Norrholm, S.D., Olin, I., Jones, M., Leimbach, L., Daugherty, M., Crowe, C., Skelton, K., Jovanovic, T., Ressler, K., Bradley, B., & Duncan, E. (September 2009). Conditioned Fear Acquisition, Discrimination, and Extinction in Combat Veterans from Operation Iraqi Freedom with Post-traumatic Stress Disorder. 3rd Annual Military Health Research Forum, Kansas City, MO.

Section V: Conclusion

The current study provides further validation for Fear Acquisition, Extinction, and Stimulus Generalization paradigms to be used as an assessment tool for fear processing in PTSD. The paradigms allow us to detect both physiological and cognitive measures of learned fear and extinguished fear, as well as generalization of danger cues. The goal of many exposure therapies (which are similar to extinction training) is to facilitate the extinction of fear acquired during combat exposure. This fear potentiated startle paradigm may prove useful for assessing the ability to extinguish learned fear both before and after exposure therapy.

Based on the data collected this far, we plan to continue as proposed in our Statement of Work and pending EWOF.

Section VI: References

Blechert, J., T. Michael, N. Vriends, J. Margraf, and F. H. Wilhelm, 2007: Fear conditioning in posttraumatic stress disorder: Evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behav Research and Ther*, **45,** 2019-2033.

Davis, M., 2002: Role of NMDA receptors and MAP kinase in the amygdala in extinction of fear: clinical implications for exposure therapy. *Eur J Neurosci*, **16,** 395-398.

Davis, M., K. Ressler, B. O. Rothbaum, and R. Richardson, 2006: Effects of d-cycloserine on extinction: translation from preclinical to clinical work. *Biol Psychiatry*, **60,** 369-375.

Friedman, M., 2006: *Post-traumatic and acute stress disorders. The latest assessment and treatment strategies.* 4th ed. Dean Psych Press Corporation.

Guthrie, R. M., and R. A. Bryant, 2006: Extinction learning before trauma and subsequent posttraumatic stress. *Psychosom Med*, **68,** 307-311.

Hofmann, S. G., and Coauthors, 2006: Augmentation of exposure therapy with d-cycloserine for social anxiety disorder. *Arch Gen Psychiatry*, **63,** 298-304.

Jovanovic, T., S. D. Norrholm, N. Q. Blanding, M. Davis, E. Duncan, B. Bradley, and K. J. Ressler, 2010a: Impaired fear inhibition is a biomarker of PTSD but not depression. *Depr Anx*, **27,** 244-251.

——, 2009: Posttraumatic stress disorder may be associated with impaired fear inhibition: relation to symptom severity. *Psychiatry Res*, **167,** 151-160.

——, 2010b: Fear potentiation is associated with hypothalamic pituitary-adrenal axis function in PTSD. *Psychoneuroendocrinology*, **35,** 846-857.

Milad, M. R., S. P. Orr, N. B. Lasko, Y. Chang, S. L. Rauch, and R. K. Pitman, 2008: Presence and acquired origin of reduced recall for fear extinction in PTSD: Results of a twin study. *J Psychiatric Res*, **42,** 515-520.

Milad, M. R., and Coauthors, 2009: Neurobiological Basis of Failure to Recall Extinction Memory in Posttraumatic Stress Disorder. *Biol Psychiatry*, **66,** 1075-1082.

Norrholm, S. D., and T. Jovanovic, 2010: Tailoring therapeutic strategies for treating posttraumatic stress disorder symptom clusters. *Neuropsychiatric Dis Treat*, **in press**.

Norrholm, S. D., T. Jovanovic, B. Vervliet, K. M. Myers, M. Davis, B. O. Rothbaum, and E. J. Duncan, 2006: Conditioned fear extinction and reinstatement in a human fear-potentiated startle paradigm. *Learn Mem*, **13,** 681-685.

Norrholm, S. D., T. Jovanovic, I. W. Olin, L. Sands, I. Karapanou, B. Bradley, and K. J. Ressler, 2010a: Fear extinction in traumatized civilians with posttraumatic stress disorder: relation to symptom severity. *Biol Psychiatry*, 69(6):556-63.

Norrholm, S.D., Olin, I.W., Jovanovic, T., McCarthy, A.J., Duncan, E.J., Skelton, K., & Bradley, B. 2010b: Conditioned fear extinction is impaired in combat veterans from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). *Proceedings of the 27th Army Science Conference*.

Norrholm, S. D., and Coauthors, 2008: Timing of extinction relative to acquisition: a parametric analysis of fear extinction in humans. *Behav Neurosci*, **122,** 1016-1030.

Ressler, K. J., and Coauthors, 2004: Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. *Arch Gen Psychiatry*, **61,** 1136-1144.

Ressler, K.J., Mercer, K.B., Bradley, B., Jovanovic, T., Mahan, A., Kerley, K., Norrholm, S.D., Kilaru, V., Smith, A.K., Myers, A.J., Ramirez, M., Engel, A., Hammack, S.E., Toufexis, D., Braas, K.M., Binder, E.B., & May, V. (2011). Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. Nature, 470(7335):492-7.

Rothbaum, B. O., and M. Davis, 2003: Applying learning principles to the treatment of post-trauma reactions. *Ann NY Acad Sci,* **1008,** 112-121.